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Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

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(88) Date of publication of the international search report:
29 January 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IDENTIFICATION OF VIRULENCE ASSOCIATED REGIONS RD1 AND RD5 ENABLING THE DEVELOPMENT OF IMPROVED VACCINES OF *M. BOVIS* BCG AND *M. MICROTI*

(57) Abstract: The present invention relates to a strain of *M. bovis* BCG or *M. microti*, wherein said strain has integrated part or all of the RD1 region responsible for enhanced immunogenicity of the tubercle bacilli, especially the ESAT-6 and CFP-10 genes. These strains will be referred as the *M. bovis* BCG::RD1 or *M. microti*::RD1 strains and are useful as a new improved vaccine for preventing tuberculosis and as a therapeutical product enhancing the stimulation of the immune system for the treatment of bladder cancer. These strains are also useful for the expression and presentation of heterologous antigens and molecule that are of therapeutic or prophylactic interest.



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INTERNATIONAL SEARCH REPORT

International Patent No. 0021
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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N1/36 A61K39/04 G01N33/56 C12N15/11 C07K14/35

Re PTO 01 OCT 2004

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EMBL, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAHAIRAS G G ET AL: "Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis." JOURNAL OF BACTERIOLOGY. UNITED STATES MAR 1996, vol. 178, no. 5, March 1996 (1996-03), pages 1274-1282, XP000647583 ISSN: 0021-9193 cited in the application abstract page 1278, right-hand column, paragraph 4 -page 1280, right-hand column, paragraph 2 page 1281, right-hand column, paragraph 2 -page 1282, left-hand column, paragraph 1 table 3	1-19, 21-24, 26, 28-48, 57-59
A	-& DATABASE NCBI 'Online! NCBI; 27 April 1996 (1996-04-27) MAHAIRAS G. ET AL.: "Mycobacterium bovis -/--	1-19, 21-24, 26,

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
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- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

3 September 2003

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	deleted region 1, 6 kDa early secretory antigenic target (esat6) gene, complete cds" XP002209499 the whole document	28-49, 57-59
X	WO 99 04005 A (STATENS SERUMINSTITUT ;ANDERSEN PETER (DK); RASMUSSEN PETER BIRK () 28 January 1999 (1999-01-28)	1-7, 15-19, 21-23, 29-33, 42-48, 57,58 49
Y	page 1, paragraph 2 page 5, paragraph 7 -page 6, paragraph 1 page 17, paragraph 2 page 44, paragraph 6 -page 46, paragraph 4 claims 12,48-53	
X	-& DATABASE EMBL 'Online! EBI; 7 June 1999 (1999-06-07) ANDERSEN P ET AL.: "Regulatory polynucleotide containing M. tuberculosis lhp/orf1c operons" XP002209498 the whole document	1-7, 15-19, 21-23, 29-33, 42-48, 57,58
X	BERTHET F X ET AL: "A Mycobacterium tuberculosis operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10)." MICROBIOLOGY (READING, ENGLAND) ENGLAND NOV 1998, vol. 144 (Pt 11), November 1998 (1998-11), pages 3195-3203, XP002901305 ISSN: 1350-0872 abstract page 3198, right-hand column, paragraph 3 -page 3199, left-hand column, paragraph 1 page 3202, right-hand column, paragraph 2 -& DATABASE EMBL 'Online! EBI; 29 June 1998 (1998-06-29) BERTHET F. ET AL.: "Mycobacterium tuberculosis H37Rv esat6 promotor region, L45 antigen homologous protein LHP (lhp) gene,complete cdc, and early secreted antigenic target 6 kDa (esat6) gene, partial cds" XP002209500 the whole document	1-7, 15-19, 21-23, 29-33, 42-45, 57,58
A		1-19, 21-24, 26, 28-49, 57-59

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IB 03/01789

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 99 45119 A (FOLKERSEN JOERGEN ;STATENS SERUMINSTITUT (DK); JENSEN CARSTEN LOEV) 10 September 1999 (1999-09-10)</p> <p>page 22, paragraph 2 -page 23, paragraph 1 page 14, paragraph 2 -page 15, paragraph 3 page 17, paragraph 4 -page 18, paragraph 1 page 29, paragraph 2 page 31, paragraph 2 example 3.2 claims 47-49</p>	<p>1-7, 15-19, 21-23, 29-33, 42-48, 57,58</p>
Y	<p>ZHANG YE ET AL: "The signalling pathway for BCG-induced interleukin-6 production in human bladder cancer cells." BIOCHEMICAL PHARMACOLOGY, vol. 63, no. 2, 2002, pages 273-282, XP001098404 15 January, 2002 ISSN: 0006-2952 page 273, left-hand column, paragraph 1 -right-hand column, paragraph 2 abstract</p>	<p>49</p>
A	<p>MUSTAFA A S: "Biotechnology in the development of new vaccines and diagnostic reagents against tuberculosis." CURRENT PHARMACEUTICAL BIOTECHNOLOGY. NETHERLANDS JUN 2001, vol. 2, no. 2, June 2001 (2001-06), pages 157-173, XP001098143 ISSN: 1389-2010 abstract page 159, left-hand column, paragraph 3 -right-hand column, paragraph 2 page 160, left-hand column, paragraph 2 -right-hand column, paragraph 1 page 162, right-hand column, paragraph 2 -page 165, left-hand column, paragraph 1</p>	<p>1-19, 21-24, 26, 28-49, 57-59</p>
A	<p>BROSCH R ET AL: "A new evolutionary scenario for the Mycobacterium tuberculosis complex." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 99, no. 6, 19 March 2002 (2002-03-19), pages 3684-3689, XP002208996 http://www.pnas.org March 19, 2002 ISSN: 0027-8424 cited in the application page 3685, right-hand column, paragraph 5 page 3688, left-hand column, paragraph 3</p>	<p>1-19, 21-24, 26, 28-49, 57-59</p>

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INTERNATIONAL SEARCH REPORT

International Application No.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BROSCH ROLAND ET AL: "The evolution of mycobacterial pathogenicity: Clues from comparative genomics."</p> <p>TRENDS IN MICROBIOLOGY,</p> <p>vol. 9, no. 9, September 2001 (2001-09),</p> <p>pages 452-458, XP002208997</p> <p>ISSN: 0966-842X</p> <p>abstract</p> <p>page 455, left-hand column, paragraph 2</p> <p>-right-hand column, paragraph 2</p> <p>table 1</p> <p>-----</p>	<p>1-19,</p> <p>21-24,</p> <p>26,</p> <p>28-49,</p> <p>57-59</p>

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International application No.
PCT/IB 03/01789

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-19, 21-24, 29-49, 57-59 (all in part) and 26 and 28 (complete)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/IB 03 01789

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: 1-19, 21-24, 29-49,
57-59 (all in part) and 26 and 28 (complete)

Strain of *M. bovis* BCG or *M. microti* transformed with a DNA fragment comprising the entire RD1 region (RD1-2F9) according to SEQ ID NO: 1 or part of it, method for the preparation thereof, cosmid and plasmids comprising said fragment and their use for the transformation of *M. bovis* BCG or *M. microti*, the corresponding DNA fragment, pharmaceutical compositions, vaccines and products comprising said strain and use of said strain for preparing a medicament or vaccine for preventing or treating tuberculosis or as adjuvant/immunomodulator for preparing a medicament for the treatment of superficial bladder cancer.

Invention 2: 1-19, 21-23,
29-49 and 57-59 (all in part)

Strain of *M. bovis* BCG or *M. microti* transformed with a DNA fragment of the RD1 region according to SEQ ID NO: 2 or part of it, method for the preparation thereof, cosmid and plasmids comprising said fragment and their use for the transformation of *M. bovis* BCG or *M. microti*, the corresponding DNA fragment, pharmaceutical compositions, vaccines and products comprising said strain and use of said strain for preparing a medicament or vaccine for preventing or treating tuberculosis or as adjuvant/immunomodulator for preparing a medicament for the treatment of superficial bladder cancer.

Invention 3: 1-19, 21-24,
29-49 and 57-59 (all in part) and 25 and 27
(complete)

Strain of *M. bovis* BCG or *M. microti* transformed with a DNA fragment of the RD1 region according to SEQ ID NO: 3 or part of it, method for the preparation thereof, cosmid and plasmids comprising said fragment and their use for the transformation of *M. bovis* BCG or *M. microti*, the corresponding DNA fragment, pharmaceutical compositions, vaccines and products comprising said strain and use of said strain for preparing a medicament or vaccine for preventing or treating tuberculosis or as adjuvant/immunomodulator for preparing a medicament for the treatment of superficial bladder cancer.

INTERNATIONAL SEARCH REPORT

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 4-28: claims 1-23, 29-49, 57,
58 (all in part)

Strain of *M. bovis* BCG or *M. microti* transformed with a DNA fragment according to SEQ ID NO: 4-28 or combinations thereof, method for the preparation thereof, cosmids and plasmids comprising said fragment and their use for the transformation of *M. bovis* BCG or *M. microti*, the corresponding DNA fragment, pharmaceutical compositions, vaccines and products comprising said strain and use of said strain for preparing a medicament or vaccine for preventing or treating tuberculosis or as adjuvant/immunomodulator for preparing a medicament for the treatment of superficial bladder cancer.

Invention 29: Claims 50-56 (complete)

Method for the identification at the species level of members of the *M. tuberculosis* complex by means of markers of the RD1 and RD5 region, resp., comprising the use of primers according to SEQ ID NO: 32-40; diagnostic kit therefore comprising DNA probes and primers hybridizing to genes according to SEQ ID NO: 14-20 or DNA probes and primers according to SEQ ID NO: 32-40; diagnostic kit comprising antibodies specific for proteins encoded by SEQ ID NO: 15, 16, 17 and/or 18.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Publication No

PCT/JP03/01789

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9904005	A	28-01-1999	AU 8123898 A	10-02-1999
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